

**Amino Acid and Vitamin Content  
of  
Selected Poultry Feedstuffs Produced in Hawaii**

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and  
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1862-1962

LAND-GRANT COLLEGES

CENTENNIAL OF THE MORRILL ACT OF 1862  
CREATING THE LAND-GRANT COLLEGE SYSTEM

## CONTENTS

	PAGE
INTRODUCTION . . . . .	3
REVIEW OF LITERATURE . . . . .	3
EXPERIMENTAL . . . . .	4
Sampling . . . . .	4
Methods . . . . .	4
RESULTS AND DISCUSSION . . . . .	6
REFERENCES . . . . .	16

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# **Amino Acid and Vitamin Content of Selected Poultry Feedstuffs Produced in Hawaii**

**A. L. Palafox and Della F. Reid**

## **INTRODUCTION**

Feedstuffs of tropical origin have been used for some time in poultry feeds on an empirical basis since adequate analytical data of their nutritive content were not available.

The present study determines the supplementary value of certain feed ingredients by analyzing their amino acid and vitamin content. Such an analysis makes possible the direct evaluation of the feeds as specific nutrient sources for the formulation of mixed diets. This information also facilitates the determination of the biological value of the feedstuffs by enabling the investigator to take into consideration nutrient imbalances or deficiencies. For example, previous studies at this station (unpublished) showed that local tuna meal proved unsatisfactory for chick growth when fed above the 9 percent level. The present study indicates that certain amino acids in this feedstuff are present in very low amounts. Much time and expense could have been spared if such information had been available prior to the tuna meal studies.

## **REVIEW OF LITERATURE**

Shorey (1906) analyzed the composition of some Hawaii feedstuffs such as algaroba, alfalfa, and sugar cane top meals for protein, fat, and fiber. He found that the proximate analyses of locally produced products and those produced elsewhere were not much different, except for the calcium content.

Palafox and Quisenberry (1949) and Olaivar (1957) have reported the use of koa haole (*Leucaena glauca*) in poultry feeds as a substitute for alfalfa meal. The value of pigeon peas for pigeons and laying hens was studied by Krauss (1932) and Draper (1944). Draper (1944) also found that algaroba meal was of value in feeding pullets, while Bice (1933) tested papayas with laying pullets. Draper (1944) found that algaroba meal promoted fair egg production with pullets.

Molasses yeast, dried *Torulopsis utilis* grown on a molasses substrate, has also been tested with poultry. Bice (1942), Klose and Fevold (1945), Goyco and Asenjo (1947, 1949), and Palafox and Rosenberg (1960) have successfully used molasses yeast with growing and laying chickens.

Scott (1953) extensively reviewed the literature dealing with the use of molasses in poultry feeds. In contrast to the lower levels of molasses used by the earlier workers, Rosenberg and Palafox (1956) successfully fed high levels to poultry for 72 weeks.

## EXPERIMENTAL

### Sampling

All the feedstuffs selected for analysis were produced in Hawaii. Alfalfa meal was obtained from a field with a stand not more than one-half in bloom. Samples of sugar cane top meal were taken from cuttings not more than 4 inches below the lowest green leaf. Napier grass meal was obtained from a sample taken from 5-week growth after a previous cutting. All of the growth 3 inches above the ground was used. Koa haole leaf meal was prepared from leaves including petioles and succulent tops of growths with not more than one blossom.

Algaroba bean pods, guava pulp (by-product after the seeds and juice were mechanically removed), koa haole seeds, papaya seeds, papaya pulp (left over after the removal of edible portion and seeds), passion fruit pulp (left over after the removal of seeds and edible portion), and pigeon pea seeds were laboratory-dried in an oven before grinding. Ground samples were allowed to reach an equilibrium with atmospheric moisture before analysis. (See table 3 for final moisture content.) Locally produced cane molasses, meat and bone meal, blood meal, and tuna meal were obtained through commercial channels.

Determinations on mainland alfalfa were made for two reasons. First, because values for the nutrients being tested for mainland alfalfa are available in the literature, these values could serve as a check on the technique. Second, a comparison was made possible on the nutritive value between local and mainland alfalfa. Since age of sample is known to have an effect on nutrient content, determinations were made on fresh materials wherever possible.

### Methods

Microbiological and chemical methods were used to determine the amino acid and vitamin content of these feedstuffs. Tables 1 and 2 show the dilutions for the amino acid and vitamin standard solutions. All analyses were calculated on the nutrient content of the feedstuffs as they are usually used in formulation of poultry rations.

The microbiological assay is based on the fact that certain microorganisms have specific amino acid and vitamin requirements for growth. When

a basal medium, complete in all requirements except for the amino acid or vitamin being tested, is used, the growth response of the organism can be compared quantitatively in standard and unknown solutions. As the organism grows, the acid produced as a metabolic product, or the turbidity resulting from increased number of cells, is proportional to the amount of test substances present. The acidity or turbidity can then be measured by standard procedures.

Microbiological procedures were preferred over chemical methods even when the latter were available for reasons of economy and convenience. The former possess the advantage of speed and small requirements of space, labor, and materials. Microbiological techniques have the additional advantage of biological specificity.

Vitamin A was determined chemically, using the Carr-Price color method. Carotene was determined by a chromatographic technique based upon the separation of the biologically active carotenoid pigments. Detailed procedures may be found in the *Methods of Vitamin Assay*, Second Edition (AOVC, 1951).

All materials were initially screened for vitamin D activity by using a modification of the biological method of the Association of Official Agricultural Chemists (1950). Five percent of each test material was incorporated into a vitamin D-free diet and fed to day-old New Hampshire chicks for 3 weeks. At the end of this time, the animals were sacrificed, and the middle toe of the left foot was ashed at 850° C for 1 hour. The toe ash of the chicks receiving the test diets was then compared with standard toe ash values obtained by feeding graded levels of USP vitamin D<sub>3</sub> reference standard. The only feedstuffs which showed any apparent vitamin D activity were meat and bone meal and tuna meal, while alfalfa meal showed a trace.

Since the high levels of calcium and phosphorus in tuna and meat and bone meals could have affected the toe ash of the groups fed these supplements, an attempt was made to extract the fat-soluble vitamin D. One pound of each meal was extracted in a soxhlet apparatus for 24 hours with ethanol, followed by 24-hour extraction with anhydrous ethyl ether. The solvents were distilled off and the resulting extracts pooled and added to 20 pounds of the vitamin D-free ration. Feed containing graded levels of the USP vitamin D<sub>3</sub> reference standard was also prepared. The feed containing the test materials as well as the control feeds were fed for 3 weeks to lots of 15 chicks each which had been previously fed the vitamin D-deficient diet for 1 week. The toes were ashed as previously described and a standard curve was constructed. The results of this test showed that the fat extract of tuna meal contained  $\frac{1}{3}$  of the vitamin D activity noted previously. The lack of any response of the fat extract from meat and bone meal indicated that the apparent vitamin D activity noted earlier must have been due to the high calcium and phosphorus content of the meal.

Protein analysis was based on the microkjeldahl method of the Association of Official Agricultural Chemists (1950). Moisture analyses were determined by drying duplicate samples to constant weight in an oven at 100° C. The loss in weight was reported as moisture content.

The microbiological techniques used are described by the AOVC (1951) and Williams (1955). Of the general methods presented for each of the nutrients, those found in tables 7 and 8 were found to be most satisfactory.

## RESULTS AND DISCUSSION

Tables 1 and 2 show the standard solutions used in this study. The L-isomers of the amino acids were used since the D-isomers are completely inactive for phenylalanine, threonine, and valine. DL-isoleucine and DL-leucine are unsatisfactory standards because of the partial activity of the D-forms. The authors found that the extraction procedures and concentrations shown in table 2 gave consistent results.

TABLE 1. Amino acid standard solutions

AMINO ACID <sup>1</sup>	CONCENTRATED STANDARD <sup>2</sup>		DILUTION		
	Weight (mg)	Make to volume (ml)	Concentrated standard (ml)	Final volume (ml)	Final concentration of standard solution (mcg/ml)
L-arginine HCl	483	200	5	500	20
L-cystine <sup>3</sup>	100	200	5	500	5
L-histidine HCl	270	200	5	500	10
L-isoleucine	400	200	5	500	20
L-leucine	400	200	5	500	20
L-lysine HCl	1004	200	5	500	40
L-methionine	200	200	5	500	10
L-phenylalanine	400	200	5	500	20
L-threonine	400	200	5	500	20
L-tryptophan <sup>3</sup>	80	200	5	500	4
L-tyrosine <sup>3</sup>	400	200	5	500	20
L-valine	400	200	5	500	20

<sup>1</sup>Amino acids were initially dissolved with water, unless specified, and made to volume with distilled water.

<sup>2</sup>The DL-form may be used by using twice the weight of the L-form.

<sup>3</sup>Initial solvent is 4 ml 2N HCl for L-cystine, 1 ml 1N NaOH each for L-tryptophan and L-tyrosine.



TABLE 2. Vitamin standard solutions

VITAMIN	CONCENTRATED STANDARD				FINAL CONCENTRATION OF WORKING STANDARD SOLUTION (mmcg/ml)
	Weight (mg)	Initial solvent (ml)	Make to volume (ml)	Concen- tration (mcg/ml)	
D-biotin (free acid)	25.00	400 50% ethyl alcohol	500	50	.20
Folic acid	100.00	5 .01N NaOH 100 20% etha- nol	100	1000	.20
Niacin	100.00	500 50% ethyl alcohol	500	200	200.00
Pantothenic acid <sup>1</sup>	50.00	500 50% ethyl alcohol	500	100	500.00
Riboflavin	50.00	1500 water <sup>2</sup> 2.4 glacial acetic acid	200	250	100.00
Vitamin B <sub>12</sub>	0.01	50 water	100	0.1	1.00

<sup>1</sup>Derived from 54.4 mg calcium pantothenate.<sup>2</sup>Warm to aid solution.

The moisture and protein content of selected feedstuffs are shown in table 3. The protein content of the materials tested is comparable to published values except for guava pulp meal, koa haole seed meal, papaya seed meal, papaya pulp meal, and passion fruit pulp meal for which no values have been found.

It is noteworthy that Hawaiian alfalfa meal contained 25.9 percent protein compared to 20.3 percent for mainland alfalfa meal. Tuna meal, blood meal, meat and bone meal, and molasses yeast are high in protein and have been used as protein concentrates in poultry rations. Alfalfa meal, koa haole leaf meal, koa haole seed meal, papaya seed meal, and pigeon pea seed meal are relatively high in protein and may have potential value as protein supplements.

On the other hand, several of the feedstuffs are low in protein (algaroba bean meal, cane molasses, guava pulp meal, napier grass meal, papaya pulp meal, passion fruit pulp meal, and pineapple bran) but may have feeding value as sources of carbohydrates, minerals, and vitamins.

The data shown in tables 4 and 5 provide a ready reference for the amino acid and vitamin content of locally produced feedstuffs. These data make it possible to compare locally produced feedstuffs for 12 amino acids and 8 vitamins.

TABLE 3. Moisture and protein control of selected feedstuffs produced in Hawaii

MATERIAL	MOISTURE %		CRUDE PROTEIN %	
	Av.	Range	Av.	Range
Alfalfa meal, Mainland	10.3	10.2-10.3	20.3	19.1-21.5
Alfalfa meal, Hawaii	9.0	8.9- 9.1	25.9	25.9-26.0
Algaroba bean meal	6.5	5.6- 7.3	10.0	9.9-10.1
Sugar cane top meal	4.1	3.0- 5.1	5.0	5.0- 5.1
Cane molasses	25.7	21.5-29.9	2.9	2.6- 3.2
Guava pulp meal	8.5	8.3- 8.6	7.6	7.3- 7.9
Koa haole leaf meal	6.3	6.2- 6.4	19.5	19.1-19.9
Koa haole seed meal	9.3	8.2-10.4	22.6	21.9-23.3
Meat and bone meal, Hawaii	8.1	7.9- 8.2	48.6	47.7-49.5
Napier grass meal	10.2	8.8-10.3	5.0	4.9- 5.1
Papaya seed meal	8.1	7.8- 8.4	22.5	21.0-24.9
Papaya pulp meal	13.3	13.0-13.5	15.7	15.1-16.3
Passion fruit pulp meal	10.1	9.1-11.0	6.2	6.1- 6.2
Pineapple bran	9.8	9.1-10.5	3.8	3.6- 3.9
Pigeon pea seed meal	7.3	7.2- 7.3	20.4	19.7-21.0
Blood meal, Hawaii	7.8	7.0- 8.6	80.7	80.5-80.8
Tuna meal, Hawaii	8.4	8.3- 8.4	61.6	57.6-65.5
Molasses yeast <sup>1</sup>	8.7	7.9- 9.4	46.5	46.3-46.7

<sup>1</sup>Dried torula yeast grown in a substrate of cane final molasses.

The amino acid content of alfalfa meal, meat and bone meal, and blood meal is consistent with published values of mainland products (Block and Weiss, 1956) with one exception. The cystine values of these products, as well as those of most of the other materials tested, are consistently low. Although the reason for this is not clear, it is possible that significant losses may have occurred during hydrolysis. Block and Weiss (1956) report numerous instances where destruction of cystine may occur.

Blood meal is a very rich source of arginine, histidine leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. On the other hand, tuna meal is low in arginine, histidine, methionine, phenyla-

lanine, and tryptophan. Blood meal may be a good feed ingredient to supplement the amino acid deficiencies of tuna meal.

The values for niacin, riboflavin, and pantothenic acid in blood meal, meat and bone scrap, and tuna meal are in general agreement with published values (Nopco, 1961). Among the plant products, alfalfa and koa haole meals are the richest sources of vitamins, although many of the other feedstuffs also provide measurable quantities of these vitamins. Passion fruit pulp meal appears to be an especially rich source of pantothenic acid and niacin.

Variable results were obtained in carotene content between samples of alfalfa meal and pineapple bran. Upon investigation, a considerable difference in age of the samples was found. Table 6 summarizes these data and emphasizes the unstable nature of this provitamin during storage.

TABLE 4. Amino acid content of selected feedstuffs produced in Hawaii

MATERIAL	ARGININE %		CYSTINE %		HISTIDINE %		ISOLEUCINE %		LEUCINE %		LYSINE %	
	Av.	Range	Av.	Range	Av.	Range	Av.	Range	Av.	Range	Av.	Range
Alfalfa meal, Mainland	.79	.77-.80	.05	.04-.05	.23	.20-.25	1.06	1.03-1.06	1.34	1.31-1.36	.85	.81-.88
Alfalfa meal, Hawaii	1.13	1.07-1.19	.04	.03-.05	.51	.49-.53	.86	.83-.88	.72	.63-.81	.86	.82-.90
Algaroba bean meal	.44	.41-.46	.03	.02-.04	.22	.18-.26	.39	.39-.39	.51	.50-.52	.30	.26-.34
Sugar cane top meal	.25	.21-.28	.01	.007-.009	.27	.22-.33	.15	.10-.18	.33	.24-.41	.29	.20-.37
Cane molasses <sup>1</sup>	-	-	-	-	-	-	-	-	-	-	-	-
Guava pulp meal	.51	.40-.71	.09	.07-.10	.29	.28-.29	.25	.20-.29	.37	.29-.44	.05	.04-.06
Koa hole leaf meal	.90	.79-1.01	.06	.05-.07	.47	.38-.56	2.09	1.06-1.12	1.74	1.59-1.89	.73	.72-.74
Koa haole seed meal	1.73	1.61-1.84	.13	.11-.14	1.40	1.03-1.77	.74	.65-.83	.96	.92-1.00	1.17	1.14-1.19
Meat and bone meal, Hawaii	4.30	4.25-4.35	.16	.15-.17	.78	.71-.85	1.23	1.20-1.26	2.68	2.60-2.76	2.39	2.19-2.59
Napier grass meal	.19	.18-.20	.02	.01-.02	.17	.15-.19	.42	.30-.54	.56	.42-.72	.28	.18-.38
Papaya seed meal	1.15	1.07-1.22	.24	.15-.33	.27	.22-.33	.43	.33-.52	.54	.45-.63	1.60	1.26-1.93
Papaya pulp meal	.13	.11-.14	.04	.037-.043	.10	.09-.11	.29	.25-.32	.42	.35-.48	1.20	1.04-1.15
Passion fruit pulp meal	.07	.06-.07	.01	.011-.013	.09	.08-.09	.07	.05-.09	.20	.19-.21	.64	.50-.77
Pineapple bran	.07	.04-.10	.01	.0091-.0093	.04	.02-.06	.14	.10-.17	.22	.19-.24	.08	.06-.12
Pigeon pea seed meal	1.50	1.46-1.60	.07	.04-.10	.89	.84-.93	.93	.73-1.12	1.39	1.29-1.49	1.11	1.01-1.20
Blood meal, Hawaii	3.27	3.15-3.39	.34	.31-.36	6.65	6.18-7.11	.99	.97-1.00	9.13	9.06-9.20	7.48	6.83-8.12
Tuna meal, Hawaii	.38	.32-.36	.41	.36-.48	.78	.60-.96	3.31	3.28-3.33	4.07	3.98-4.16	4.83	4.81-4.84
Molasses yeast <sup>2</sup>	1.70	1.57-1.83	.11	.10-.12	1.34	1.22-1.46	1.97	1.88-2.06	1.16	1.15-1.17	2.59	2.32-2.85

(Continued)

TABLE 4. Amino acid content of selected feedstuffs produced in Hawaii (Continued)

MATERIAL	METHIONINE %		PHENYLALANINE %		THREONINE %		TRYPTOPHAN %		TYROSINE %		VALINE %	
	Av.	Range	Av.	Range	Av.	Range	Av.	Range	Av.	Range	Av.	Range
Alfalfa meal, Mainland	.15	.13-.17	.80	.75-.84	.93	.91-.94	.31	.28-.33	.69	.68-.69	1.22	1.21-1.24
Alfalfa meal, Hawaii	.06	.04-.08	.80	.78-.82	.95	.93-.97	.33	.30-.35	.64	.60-.67	1.01	.94-1.08
Algaroba bean meal	.04	.03-.05	.30	.27-.32	.26	.23-.29	.07	.06-.08	.31	.29-.33	.55	.40-.69
Sugar cane top meal	.02	.02-.02	.31	.24-.37	.18	.12-.23	.08	.06-.09	.33	.30-.36	.27	.24-.31
Cane molasses <sup>1</sup>	-	-	-	-	-	-	-	-	-	-	-	-
Guava pulp meal	.02	.01-.02	.23	.19-.27	.18	.17-.18	.05	.04-.06	.37	.36-.38	.32	.27-.37
Koa haole leaf meal	.18	.16-.19	.85	.84-.85	.69	.68-.70	.42	.40-.44	.99	.80-1.18	1.51	1.21-1.81
Koa haole seed meal	.10	.09-.10	.77	.76-.78	.75	.69-.80	.21	.20-.22	.56	.50-.61	1.29	.87-1.70
Meat and bone meal, Hawaii	1.14	.91-1.33	2.83	2.40-3.25	1.78	1.65-1.90	.33	.31-.35	.85	.61-1.08	2.54	2.50-2.57
Napier grass meal	.13	.12-.14	.66	.60-.71	.34	.32-.35	.05	.04-.06	.44	.40-.48	.53	.40-.65
Papaya seed meal	.06	.05-.07	.36	.33-.39	.45	.44-.45	.05	.04-.05	.84	.68-1.00	.53	.45-.61
Papaya pulp meal	.04	.03-.04	.93	.82-1.03	.24	.22-.25	.12	.10-.13	.59	.56-.62	.51	.50-.51
Passion fruit pulp meal		trace	.28	.27-.28	.12	.11-.13	.03	.02-.04	.23	.22-.24	.21	.20-.22
Pineapple bran	.02	.01-.02	.34	.30-.37	.13	.12-.14	.12	.10-.14	.27	.22-.32	.17	.16-.17
Pigeon pea seed meal	.18	.12-.23	.14	.12-.16	.83	.79-.87	.15	.14-.16	.63	.60-.65	1.98	1.96-1.99
Blood meal, Hawaii	1.55	1.40-1.70	4.90	4.73-5.07	4.59	4.38-4.79	1.37	1.28-1.45	1.47	1.45-1.48	8.02	7.05-8.99
Tuna meal, Hawaii	.30	.26-.33	1.98	1.80-2.16	2.81	2.60-3.01	.37	.35-.40	1.57	1.25-1.86	3.35	3.29-3.40
Molasses yeast <sup>2</sup>	.46	.45-.47	2.15	2.10-2.18	2.40	2.00-2.80	.58	.57-.60	1.39	1.36-1.42	2.56	2.53-2.59

<sup>1</sup>Cane molasses not analyzed for amino acids.<sup>2</sup>Dried torula yeast grown in a substrate of cane final molasses.

TABLE 5. Vitamin content of selected feedstuffs

MATERIAL	NIACIN MICROGRAM/GRAM		RIBOFLAVIN MICROGRAM/GRAM		PANTOTHENIC ACID MICROGRAM/GRAM		BIOTIN MICROGRAM/GRAM	
	Av.	Range	Av.	Range	Av.	Range	Av.	Range
Alfalfa meal	33.60	24.43- 39.28	12.82	12.20 -13.82	19.60	17.91-22.49	.34	.27-.39
Algaroba bean meal	28.10	23.37- 34.06	1.54	1.44 - 1.63	8.65	7.75- 9.41	.13	.11-.15
Sugar cane top meal	19.09	14.84- 25.45	3.50	3.23 - 3.86	23.52	13.69-32.06	.23	.17-.26
Cane molasses	17.10	16.35- 18.25	2.60	2.01 - 3.33	50.60	48.66-52.66	.45	.41-.50
Guava pulp meal	17.01	12.62- 20.21	.44	.405- .530	13.06	11.16-14.83	.02	.01-.02
Koa haole leaf meal	55.59	42.75- 72.25	14.26	13.07 -15.58	11.69	8.25-16.50	.58	.50-.65
Koa haole seed meal	13.14	6.45- 17.62	2.68	2.16 - 3.26	10.48	9.49-11.24	.20	.18-.22
Meat and bone meal, Hawaii	44.70	35.35- 56.43	5.99	3.32 - 7.65	5.98	5.66- 6.33	.31	.30-.32
Napier grass meal	20.50	18.71- 23.06	3.07	2.34 - 3.79	14.59	9.06-21.67	.34	.28-.37
Papaya seed meal	18.67	14.75- 23.44	2.85	2.70 - 2.99	6.79	5.71- 7.71	.13	.11-.14
Papaya pulp meal	33.58	29.44- 35.69	2.48	2.37 - 2.56	6.00	3.80- 8.50	.12	.11-.12
Passion fruit pulp meal	56.94	51.13- 63.12	4.63	4.33 - 4.88	86.44	84.99-87.90	.02	.01-.02
Pineapple bran	10.58	5.95- 13.75	3.06	2.93 - 3.18	18.31	14.34-21.67	.06	.05-.07
Pigeon pea seed meal	18.03	9.54- 26.10	1.42	1.30 - 1.55	21.22	18.34-22.92	.24	.22-.26
Blood meal, Hawaii	35.39	31.87- 39.31	.45	.418- .506	2.86	2.42- 3.09	.03	.02-.03
Tuna meal, Hawaii	173.1	162.5 -181.5	4.82	4.21 - 5.54	4.87	3.58- 5.83	.39	.37-.40

(Continued)

TABLE 5. Vitamin content of selected feedstuffs (Continued)

MATERIAL	FOLIC ACID MICROGRAM/GRAM		VITAMIN B <sub>12</sub> MILLIMICROGRAM/GRAM		CAROTENE <sup>1</sup> MICROGRAM/GRAM		VITAMIN A <sup>2</sup> MCG/GRAM		VITAMIN D ICU/GRAM	
	Av.	Range	Av.	Range	Av.	Range	Av.	Range	Av.	Range
Alfalfa meal	3.88	3.73-4.07	3.13	2.89- 3.27	329.00	270.10-363.50			trace	
Algaroba bean meal	2.32	2.27-2.37	3.10	2.30- 4.40	1.67	1.15- 2.20			<sup>2</sup>	
Sugar cane top meal	.53	.40- .67	5.60	1.67- 7.70	17.00	16.00- 17.00			none	
Cane molasses	.12	.10- .13	2.67	2.55- 2.87	1.12	1.00- 1.30			none	
Guava pulp meal	.21	.17- .24	.79	.75- .86	1.90	1.90- 1.90			<sup>2</sup>	
Koa haole leaf meal	.40	.33- .48	5.03	4.15- 5.75	470.00	465.00-475.00			none	
Koa haole seed meal	1.04	.90-1.17	2.58	1.62- 4.30	4.42	4.38- 4.46			<sup>2</sup>	
Meat and bone meal, Hawaii	.67	.62- .72	19.79	10.87-31.40			trace		none	
Napier grass meal	.92	.72-1.05	2.37	2.02- 2.73	40.00	35.00- 46.00			none	
Papaya seed meal	.05	.04- .06	1.77	1.22- 2.72	10.95	10.40- 11.50			none	
Papaya pulp meal	.15	.14- .16	.81	.72- .87	37.36	36.74- 38.00			none	
Passion fruit pulp meal	.13	.11- .14	1.14	1.10- 1.22	0.30	0.30- 0.31			<sup>2</sup>	
Pineapple bran	.11	.09- .13	.12	.11- .12	21.08	19.80- 22.30			none	
Pigeon pea seed meal	.10	.09- .10	.44	.42- .47	1.19	1.19- 1.19			<sup>2</sup>	
Blood meal, Hawaii	.03	.02- .03	4.15	3.06- 5.92			trace		none	
Tuna meal, Hawaii	.06	.05- .07	22.06	14.01-29.06			trace		1.89	

<sup>1</sup>Blank spaces indicate that no analyses were made since animal products were not expected to contain carotene and vegetable products were not expected to contain vitamin A.<sup>2</sup>Data not available.

TABLE 6. Carotene content of fresh and old alfalfa meal and pineapple bran<sup>1</sup>

MATERIAL	KIND	REPLICATE	CAROTENE CONTENT MCG/GM
Alfalfa meal, dehydrated, Mainland	old	1	188.3
	old	2	69.6
	old	3	95.7
	Av.		117.9
Alfalfa meal, dehydrated, Mainland	fresh	1	363.5
	fresh	2	270.1
	fresh	3	353.5
	Av.		329.0
Pineapple bran	old	1	4.25
	old	2	4.25
	Av.		4.25
Pineapple bran	fresh	1	22.36
	fresh	2	19.80
	Av.		21.08

<sup>1</sup>Fresh sample, 1-2 weeks old; old sample, 6-8 months old.

TABLE 7. Methods used in the microbiological assay of amino acids

AMINO ACID <sup>1</sup>	ORGANISM <sup>2</sup>	EXTRACTION		TYPE OF MEASUREMENT
		ml/gm dry sample	Time autoclaved <sup>3</sup>	
Arginine	<i>Leuconostoc mesenteroides</i> P-60 ATCC No. 8042	20 ml 3N HCl	16 hours	Acidimetric
Cystine	" " "	" "	" "	"
Histidine	" " "	" "	" "	"
Isoleucine	" " "	" "	" "	"
Leucine	" " "	" "	" "	"
Lysine	" " "	" "	" "	"
Methionine	" " "	" "	" "	"
Phenylalanine	" " "	" "	" "	"
Tyrosine	" " "	" "	" "	"
Valine	" " "	" "	" "	"
Threonine	<i>Streptococcus faecalis</i> ATCC No. 8043	20 ml 5N NaOH	" "	"
Tryptophan	<i>Lactobacillus arabinosus</i> 17-5 ATCC No. 8014	" "	" "	"

<sup>1</sup>Dehydrated media were obtained from Difco Laboratories, Cleveland 28, Ohio.<sup>2</sup>All organisms were obtained from the American Type Culture, 2029 M Street, N. W., Washington 25, D. C.<sup>3</sup>All samples were extracted in the autoclave at 15 pounds pressure.



TABLE 8. Method used in the microbiological assay of vitamins

VITAMIN <sup>1</sup>	ORGANISM <sup>2</sup>	EXTRACTION		TYPE OF MEASUREMENT
		Extractant	Time	
Biotin	Lactobacillus arabinosus 17-5 ATCC No. 804	6N H <sub>2</sub> SO <sub>4</sub>	Autoclaved <sup>3</sup> 15 minutes	Acidimetric
Niacin	" " "	1N H <sub>2</sub> SO <sub>4</sub>	"	"
Pantothenic acid	" " "	H <sub>2</sub> O, alkaline phosphatase, chicken liver extract 0.1M NaHCO <sub>3</sub>	Incubated 4 hours at 37° C	"
Folic acid	Streptococcus lactis R 8043	M/5 phosphate buffer with chicken pancreas	Heat 5 minutes on water bath	Turbidimetric
Riboflavin	Lactobacillus casei ATCC No. 7469	0.1N HCl	Autoclaved <sup>3</sup> 15 minutes	Acidimetric
Vitamin B <sub>12</sub>	Lactobacillus leichmanii ATCC No. 7830	H <sub>2</sub> O	Heated 15 minutes on water bath	"

<sup>1</sup>Dehydrated media were obtained from Difco Laboratories, Cleveland 28, Ohio.<sup>2</sup>All organisms were obtained from the American Type Culture, 2029 M Street, N. W., Washington 25, D. C.<sup>3</sup>All samples were extracted in the autoclave at 15 pounds pressure.

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